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Viral etiology of acute respiratory diseases in Rio de Janeiro: first two years of a longitudinal study

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A two-year study was undertaken to establish the incidence and possible viral etiology of acute respiratory diseases among the child population of a shanty town in Rio de Janeiro, Brazil. The results demonstrated that nearly half of all the illnesses seen were respiratory infections, 10% of them affecting the lower respiratory tract. Viruses were isolated from 20% of the throat swabs collected. Of the viruses identified, 47% were adenoviruses, 25% were enteroviruses, 9% were influenza A, 8% herpes simplex, 7% parainfluenza, 3% respiratory syncytial and 1% influenza B viruses.

Respiratory diseases have been shown to be a major cause of both morbidity and mortality in the world. It has been estimated that approximately 2.2 million deaths occur annually because of acute respiratory infections (ARI) alone (*7*). Many studies have been done in the developed countries to determine the etiology, prevalence and seasonal variation of ARI (*1-4*), but only a few (*5, 6*) have been carried out in the developing regions of the world, where there is urgent need for this information.

In 1976, the Twenty-ninth World Health Assembly recognized that little information was available on ARI and called for the establishment of a WHO Scientific Group on Viral Respiratory Diseases. This Group, which met in 1979, recommended that research should be focused especially on epidemiological and etiological studies which were needed for the elaboration of possible control measures and/or therapy schemes (*7*).

The present paper gives the results of a two-year longitudinal study of ARI of viral etiology, which was carried out in a primary health care unit serving a shanty town population located in the city of Rio de Janeiro.

MATERIALS AND METHODS

The study covered the period from January 1980 to December 1981 and involved close collaboration between the primary health care unit of the National School of Public Health and the virology department, both of which are part of the Oswaldo Cruz Foundation, which is a research institute of the Brazilian Ministry of Health.

Study area

The study area was in Rio de Janeiro, a tropical city with an average summer temperature of 28 °C and average winter temperature of 18 °C. The relative humidity is high and the rainy season occurs during the summer.

The area served by the primary health care unit covered 3 km² and had an estimated population of 45 000, of whom 24 000 lived in shanty towns. The area comprised the Manguinhos district, including the Oswaldo Cruz Foundation campus and seven adjacent shanty towns (Amorin, Parque Joao Goulart, Vila Turismo, Democraticos 30, Ex-Combatentes, Vila Uniao, and Perereca), and part of the Bonsucesso district, a residential and commercial area with its own general hospital (Hospital Geral de Bonsucesso). While this population had the choice in selecting the health care facility that it wanted and many went to health services outside their own district, the people in the shanty towns adjacent to the health care unit mostly attended this unit only.

The health statistics on the population in these shanty towns showed high illiteracy rates, infant mortality greater than 100 per 1000 live births, low vaccination coverage, and malnutrition affecting more than half of the children above the age of two years.

Study population

Children between 0 and 5 years of age, who attended the health care unit with an acute upper or

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lower respiratory tract disease within the first 3 days of the onset of symptoms, were eligible to be included in this study. The diagnosis was made after physical examination of the patients. In a few cases, blood tests and chest X-rays were done to confirm the clinical diagnosis.

The age distribution of the patients was similar during both years of this study and closely reflected the use made of the health unit by the children. Thus, 39% of the patients from whom a throat swab was obtained were < 1 year, 28% were 1–2 years old, and 33% were 2–4 years old. The prevalence of malnutrition, as measured by weight, was high and affected 25% of the < 1-year-olds, 43% of the 1–2-year-olds, and 50–60% of children above the age of two.

Material collected

Single cough throat swabs were obtained and sent to the laboratory in virus transport media (Eagle's MEM (minimum essential medium) plus 0.2% bovine serum albumin) without antibiotics at 4 °C. Samples were inoculated into tissue cultures within 2 h of collection.

Laboratory procedures

The throat swabs were plated on 10% sheep's blood agar and, after incubation at 37 °C for 24 h, the plates were examined for the presence of beta-haemolytic streptococci. After the addition of penicillin, amphotericin B and streptomycin to the specimens, they were inoculated into sets of tubes of rhesus monkey kidney (RMK) cells, human embryo lung fibroblasts (HELF, strain MRC-5), canine kidney cells (MDCK line), and HEp-2 cells. The RMK cells[a] were used during the first passage. Occasionally, primary RMK cells were prepared in our laboratory from rhesus monkeys obtained from a monkey colony located on the Oswaldo Cruz Foundation campus.

The maintenance medium for the HEp-2 and MRC-5 cells consisted of Eagle's MEM, supplemented with 2% inactivated (56 °C for 30 min) fetal calf serum, and adjusted to pH 7.4 with 7.5% sodium bicarbonate. The RMK cells were maintained on the same medium but without the serum. The MDCK cells were used with the same medium, again lacking serum, but containing 1.5 μg/ml of crystalline trypsin[b] after prior washing of the cell monolayer with medium without serum.

Cultures were incubated at 33 °C for 21 days on a rotating drum, except for the HEp-2 cells, which were maintained in a stationary rack. The RMK cells were tested for haemadsorption using guinea pig erythrocytes at 5–7 day intervals; the MDCK culture fluids were tested for haemagglutination using guinea pig and chicken erythrocytes one week after inoculation, or before this time if the cell monolayer was destroyed.

The HEp-2 and HELF cells were examined on alternate days for any cytopathic effect. The maintenance media were also replenished at 2–4-day intervals. The procedures employed for virus identification were haemadsorption-inhibition (for parainfluenza and mumps viruses), haemagglutination-inhibition (influenza viruses A and B), and serum neutralization (enteroviruses, adenoviruses, respiratory syncytial virus, and alpha herpesviruses).

[a] Obtained from M.A. Bioproducts, Bethesda, MD, USA.

[b] BDH Chemicals Ltd, Poole, England.

RESULTS

The morbidity data were collected by various physicians at the health care unit and presented as percentages. Fig. 1 shows the comparative rates of respiratory, gastrointestinal and other diseases on a fortnightly time period. The relative importance of lower respiratory tract infections (LRTI), gastroenteritis, and childhood diseases are shown in the shaded areas. It should be noted that the 1980 data were collected by physicians working at the health care unit while the 1981 data were collected entirely by one of the authors (FS).

In 1980, 45% of the diagnoses were respiratory diseases, of which 10% were LRTI; no outbreak of ARI was apparent during this period. In 1981, 43% of the diagnoses were ARI with 12% LRTI; during the months of April and May a small increase in upper respiratory tract infections (URTI) and LRTI occurred.

Gastrointestinal diseases made up 35% and 36% of the diagnoses in 1980 and 1981, respectively, a large proportion being parasitic infections, while gastroenteritis was diagnosed in 7% and 9% respectively in the two years. Other diseases accounted for 20% and 21% of the diagnoses in 1980 and 1981, respectively. Childhood diseases increased during the second half of 1981 with a high incidence of varicella, rubella, mumps and measles.

One-third of the patients with ARI, examined by one of the authors, fitted the criteria (see above under *Study population*) and 371 throat swabs were collected for examination; 76 (20.4%) of them yielded the following viruses: 7 (9%) influenza A (H3N2), 1 (1%) influenza B, 5 (7%) parainfluenza viruses, 2 (3%) respiratory syncytial virus, 6 (8%) human (alpha) herpesvirus 1, 36 (47%) adenoviruses (types 1–7), and 19 (25%) enteroviruses (human poliovirus 3 (vaccine strains), coxsackievirus A7 and B4, and echovirus types 6, 7, 9, 11, 17, 18 and 21). Table 1

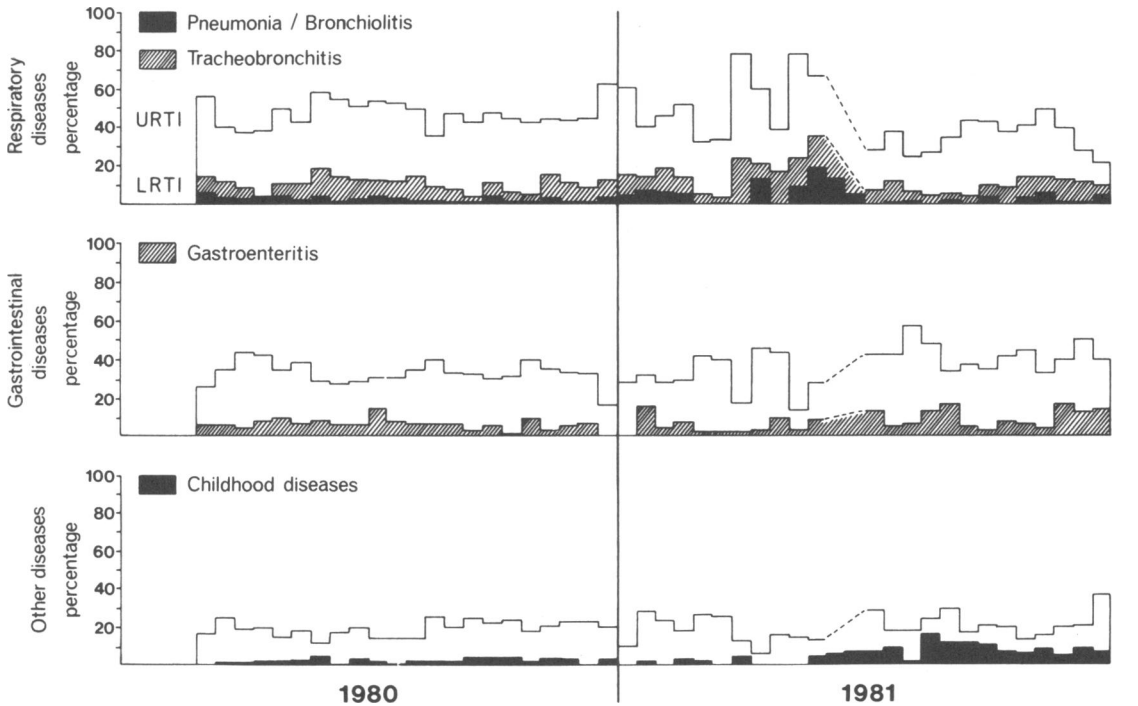

Fig. 1. Morbidity of respiratory, gastrointestinal and other diseases among patients attending a health care unit in a shanty town of Rio de Janeiro, 1980–81.

Table 1. Number and types of virus isolated from 371 throat swabs examined in 1980 and 1981

Isolates	1980	1981	Total
Influenza virus A (H3N2)	6	1	7 (9.2)[a]
Influenza virus B	0	1	1 (1.3)
Parainfluenza virus 3 and others[b]	3	2	5 (6.6)
Respiratory syncytial virus	1	1	2 (2.6)
Alpha herpesvirus 1	1	5	6 (7.9)
Adenoviruses 1–7	14	22	36 (47.4)
Enterovirus[c]	10	9	19 (25.0)
Total virus isolations	35	41	76
No. of throat swabs tested	179	192	371
Percentage of virus isolations	(19.5)	(21.3)	(20.4)

[a] Percentage out of the total viruses isolated is given in parentheses.

[b] Not typed.

[c] Poliovirus 3, coxsackievirus A7 and B4, echovirus types 6, 7, 9, 11, 17, 18, 21.

summarizes these data. In 9% of the samples, virus isolation was impossible owing to bacterial overgrowth in all the cell lines that contained penicillin, streptomycin and amphotericin B.

Fig. 2 summarizes the distribution of virus isolations according to the age of the children. The majority of the isolates were obtained from the under-3-year-olds who accounted for 70% of the paediatric consultations at the health unit.

In Table 2 the virus isolation rates, according to diagnosis, are shown. The URTI gave an overall isolation rate of 26.4%, the rate for the common cold being only 8.3%. For LRTI the overall virus isolation rate was 14.2%, with especially low rates for tracheobronchitis and pneumonia (11.3% and 14.7% respectively), compared to laryngitis and bronchiolitis (22.2% and 33.3% respectively).

Table 3 shows the frequency of different virus isolations in upper and lower respiratory tract infections, in both of which the individual types of viruses appear to be important in about the same proportions, except that the adenoviruses were much more frequent in URTI.

Influenza A viruses (H3N2) were circulating in

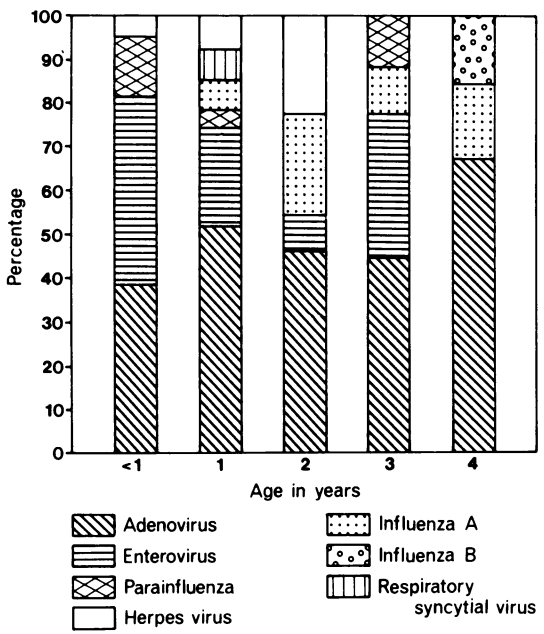

Fig. 2. Relative frequencies (%) of different viruses associated with acute respiratory diseases, by age.

the late autumn and winter of both years and were identified as antigenically intermediate between A/Texas/1/77 and A/Bangkok/1/79. In 1980 this virus was present from May to July, while in 1981 it was isolated mostly in May (with very few samples in June and July). These viruses were isolated mainly from children over 2 years old with upper and lower respiratory tract diseases (Fig. 2). In 1980 one isolate was obtained from an adult during the same period.

Influenza B virus was detected in September 1981 in a 4-year-old child with a flu-like illness, and one month later a strain was isolated from another child who did not belong to the study population. Both isolates were similar to the variant B/Singapore/222/79.

Parainfluenza virus 3 was circulating in October and November 1980 and in September and October 1981. These strains were isolated mainly from children between the ages of 6 months and 2 years. One strain, which was not fully identified, was isolated in February 1980.

Respiratory syncytial virus (RSV) was grown from two one-year-old children with LRTI. In 1980 the infection occurred in March and in 1981 in April. In 1981, during the same period, five RSV isolates were obtained from children in hospital.

The adenoviruses during both years showed no

Table 2. Virus isolation rates according to the diagnosis of infections of the upper respiratory tract (URTI) and lower respiratory tract (LRTI) in Rio de Janeiro, 1980–81

Diagnosis	1980		1981		Totals	
	No. of specimens	No. of virus isolations	No. of specimens	No. of virus isolations	No. of specimens	No. of virus isolations
URTI:						
Common cold	7	0	5	1	12	1 (8.3)[a]
Pharyngitis	24	9	52	15	76	24 (31.6)
Tonsillitis	6	2	9	1	15	3 (20.0)
Flu-like illness	52	14	38	10	90	24 (26.7)
Otitis media	6	1	9	2	15	3 (20.0)
Total	95	26 (27.4)	113	29 (25.7)	208	55 (26.4)
LRTI:						
Laryngitis	4	2	5	0	9	2 (22.2)
Tracheobronchitis	34	3	37	5	71	8 (11.3)
Bronchiolitis	3	1	3	1	6	2 (33.3)
Pneumonia	14	3	20	2	34	5 (14.7)
Total	55	9 (16.4)	65	8 (12.3)	120	17 (14.2)

[a] Figures in parentheses indicate the percentages of isolations among the specimens examined.

Table 3. Number and types of virus isolates and their relative importance in upper (URTI) and lower respiratory tract infections (LRTI) in Rio de Janeiro during 1980 and 1981

Virus[a]	URTI (208 specimens)	LRTI (120 specimens)
Influenza virus A	5 (2.4)[b]	2 (1.7)
Influenza virus B	1 (0.5)	– (–)
Adenovirus	28 (13.4)	5 (4.2)
Enterovirus	14 (6.7)	4 (3.3)
Herpesvirus	4 (1.9)	2 (1.7)
Parainfluenzavirus	3 (1.4)	2 (1.7)
Respiratory syncytial virus	– (–)	2 (1.7)
	55 (26.4)	17 (14.2)

[a] The following bacteria were also isolated: beta-haemolytic streptococci, 1; and *Escherichia coli*, 1.

[b] Figures in parentheses are percentages out of the total number in each group (URTI and LRTI).

clear seasonal pattern or age preference. Most of them were isolated from patients with upper respiratory tract infections and only a few from those with LRTI. Types 1 to 7 were identified, with type 5 predominating.

Enteroviruses were isolated in both years, with an even distribution over the year, but were apparently more frequent in the younger infants. Human poliovirus 3 (vaccine strain) was isolated after a nationwide mass vaccination with oral vaccine. Coxsackievirus B4 was found frequently and was also isolated from other throat swabs received at the laboratory. There was no predominance of any one serotype among the echoviruses that were isolated.

Human (alpha) herpesvirus 1 was isolated from one child in 1980 and from five children in 1981. Of these, four had URTI and two LRTI. Their ages varied from 6 months to 2 years.

Rhinoviruses were not isolated during these two years.

The role of bacteria was not investigated other than by a simple blood agar inoculation for the detection of haemolytic streptococci. The latter were rarely detected.

DISCUSSION

In 1980 a two-year study of acute respiratory infections was started in Rio de Janeiro to obtain data on their prevalence and possible viral etiology. Previously, studies carried out in this city were mainly during epidemics (*8*) or were short-term hospital-based studies (*9–11*), which gave little information on the true morbidity and seasonal variations.

The target population for this study was selected by taking various factors into account. Thus, we studied children under five years of age because it is known that they are the ones most affected (*7*), and we selected a locality not far from our laboratory so that the throat swabs could be transported there well within a two-hour time limit. We also wanted to study a representative population of the lower socioeconomic group living within the city limits of Rio de Janeiro. So the population we selected had an infant mortality rate of more than 100 per 1000 live births, malnutrition affected more than 50% of the children above the age of two years, the illiteracy rate was high, and the level of hygiene and sanitation was poor.

Our results showed that 41% of the patients presenting at the health care unit had respiratory infections, of which about 14% involved the lower respiratory tract. This rate is higher than those observed in other countries, e.g., in a rural medical centre in Venezuela, 27% of the consultations were due to ARI;[c] in Australia, ARI represented 32% of all diseases seen by general practitioners (*12*); and in Britain this was 28% (*13*).

During the same period, the proportion of respiratory infections in all paediatric admissions to a private hospital (Policlinica de Botafogo), which is under contract by the National Health Insurance System to supply medical care, was found to be 49.2% and 54.4% in 1980 and 1981, respectively (V. Farias, personal communication). These rates are much higher than the 21% observed in Britain (*13*). From these limited data it seems that morbidity due to respiratory diseases is very high in Rio de Janeiro.

In our study of 371 throat swabs, 76 of them yielded viruses (20.4% isolation rate), which is comparable with the results of other workers (*6, 14–16*), especially during non-epidemic periods. Table 4 summarizes the viral isolation rates found in studies in both developing and developed countries. Our results are similar to those obtained in the studies done in West Bengal, India (*15*), and in Uganda (*6*) where similar population groups were studied and where a high incidence of adenoviruses and enteroviruses was also observed. The studies done in Kuala Lumpur (*17*) and Singapore (*18*) gave higher overall isolation rates and the percentages of RSV were higher, which might indicate better specimen collection (aspirates), or that these studies were done in areas of high RSV endemicity, or that these investigations coincided with RSV outbreaks.

[c] *Acute respiratory infections in children*. Washington, Pan American Health Organization, 1982 (unpublished document RD21/3).

Table 4. Percentage isolation rates of different viruses in various studies

Country or area	Reference	No. of virus isolates/ No. of specimens	Influenza virus		Para-influenza virus	Respiratory syncytial virus	Adeno-virus	Entero-virus	Rhino-virus	Herpes-virus	*Mycoplasma pneumoniae*
			A	B							
Rio de Janeiro	–	76/371 (20.5)[a]	9.2	1.3	6.6	2.6	47.4	25.0	–	7.9	NT[b]
Kuala Lumpur	Ong et al. (*17*)	59/180 (32.8)	11.9	5.1	10.2	44.1	3.4	3.8	15.4	–	NT
Singapore	WHO (*18*)	483/1399 (34.5)	11.2	14.7	13.2	24.8	12.0	6.2	17.8	–	13.2
West Bengal	Kloene et al. (*15*)	483/4171 (11.6)	–	0.6	11.4	2.5	33.1	40.4	0.4	6.6	NT
Uganda	Soběslavský et al. (*6*)	116/662 (17.5)	–	–	13	1	33	41	–	12	NT
Calcutta	Ota & Bang (*14*)	232/1719 (13.5)	–	3.0	7.7	4.7	51.7	9.9	–	14.7	NT
Tecumsech (MI, USA)	Monto et al. (*4*)	303/1419	4.9	9.9	22.1	7.9	3.6	5.2	46.2	–	0.4

[a] Figures in parentheses are percentages.

[b] NT = not tested.

The influenza isolation rate in ARI tends to be low in most studies (*14, 17*) except during epidemics. In our study, although influenza A virus was isolated during the winter and autumn months, the isolates were few in number and a serological survey with sera obtained from a general hospital confirms the impression that the virus did not circulate with great intensity; indeed, 1980 and 1981 were years with little influenza activity in most other countries of the world (*19*). All the influenza A viruses (H3N2) isolated during 1980 and 1981 were antigenically intermediate between A/Texas/1/77 and A/Bangkok/1/79. There were no isolations of A/Brazil/11/78 (H1N1). These findings agree with the results of a serological study by Chaves et al. (*20*), which showed that antibody to virus A/Texas/ 1/77 (H3N2) was infrequent, whereas 60% of the population under the age of 20 gave evidence of immunity to virus A/Brazil/11/78 (H1N1).

The single influenza B virus isolated in the spring (September) of 1981 was similar to the variant B/Singapore/222/79. A study by Nascimento et al. (*21*) in Rio de Janeiro showed that antibodies to influenza B were widespread, having been acquired throughout childhood but particularly after children started school. This might explain the low isolation rate of influenza B in this study. Similar results have been observed in the United Kingdom (*22*).

In some laboratory studies the MDCK cell line has been shown to be as effective as RMK cells for the isolation of influenza virus (*23, 24*). In our study there was complete correlation between influenza A and B isolations with cultures of these 2 cells.

Parainfluenza virus 3 usually causes febrile illnesses in the first months of life and is frequently involved in infections of the lower respiratory tract. In our study this virus was isolated in the spring months among children aged from six months to 2 years old, some of them with LRTI.

Respiratory syncytial virus was isolated from two patients with LRTI in the autumn months (March and April). In 1981 it coincided with an increase of ARI morbidity and was detected in a few hospitalized patients. The low isolation rate may possibly be explained by the fact that, although we used a cell line known to be sensitive to this virus, the number of infected cells likely to be obtained by throat swabbing may be very few and we believe immunofluorescence on nasopharyngeal aspirates would have greatly improved the diagnostic rate.

Adenoviruses were the viruses most frequently isolated (47%) and were found continuously during the two years of our study. This rate was higher than expected although, in a previous short-term study in the same city, an isolation rate of 41% had been obtained,[d] and some authors in other countries have found similar rates (*6, 14, 15*). Serotypes 5 and 1 were the most frequent, followed by types 7, 2, 4 and (very rarely) types 3 and 6. There was no apparent periodicity among the serotypes. Adenoviruses were isolated mostly from upper respiratory tract infections (Table 3).

[d] ONO, I. P. C. M.Sc. thesis, University of Rio de Janeiro, 1977.

Enteroviruses were the second most frequent (25%) agents found. Ten echoviruses and six coxsackieviruses were isolated in both years with no clear periodicity. Two enteroviruses were not identified and the presence of vaccine strains of poliovirus after a mass vaccination with oral vaccine was not surprising.

Human herpesvirus 1 was identified in infections of the upper respiratory tract and its importance in the lower respiratory tract was not determined.

Rhinoviruses were not encountered during this study, probably owing to the fact that the cytopathogenic effect was not recognized. Rhinoviruses are usually isolated from patients with common colds, which may be another explanation for the low isolation rate, although there are some recent reports that they may be important in LRTI (*17, 18*).

Mycoplasma pneumoniae was not identified because of laboratory limitations. We assume that it is infrequent among young children, but it will be important to include a search for it in future studies.

The excessive use of antibiotics is a well recognized problem in Brazil as in other countries (*25*). A short-term benefit has been obtained at the health care unit since physicians began to use these drugs more rationally and less of them. As it is hoped that standardized clinical mangagement of ARI in children may lead to a reduction in mortality, we would like to stress that management flowcharts should also be tested especially where etiological studies are being carried out.

Essential information was obtained during this study for the planning of future projects. Further studies are now under way to extend the work with improved virological methods, including immunofluorescence for the detection of virus antigens in cells of the respiratory tract obtained by nasopharyngeal aspiration, and with the addition of bacteriological tests to determine the role of agents other than viruses in acute respiratory diseases in this part of the world.

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RÉSUMÉ

ÉTIOLOGIE VIRALE DES AFFECTIONS RESPIRATOIRES AIGUËS À RIO DE JANEIRO: LES DEUX PREMIÈRES ANNÉES D'UNE ÉTUDE LONGITUDINALE

Cette étude a été entreprise à la suite de la constatation par la Vingt-Neuvième Assemblée mondiale de la Santé en 1976 de l'insuffisance d'information sur le rôle des affections respiratoires aiguës dans la mortalité élevée qui frappe les nourrissons des pays en développement.

On a choisi une population vivant dans les bidonvilles de Rio de Janeiro pour étudier l'incidence et l'étiologie des infections aiguës de l'appareil respiratoire chez les enfants de moins de cinq ans sur une période de deux ans (1980-81). L'existence d'une unité sanitaire étroitement associée à un laboratoire équipé pour faire des études virologiques a donné la possibilité de procéder à une évaluation clinique soigneuse des cas à traiter ainsi qu'à la collecte d'échantillons pour procéder rapidement à leur analyse au laboratoire de virologie. Dans 20% des frottis de la gorge recueillis, des virus ont été identifiés, de différents types: les plus fréquents étaient les adénovirus (47%), suivis par les entérovirus (25%), le virus grippal A (9%), le virus de l'herpes simplex (8%), le virus paragrippal (7%), le virus respiratoire syncytial (3%) et le virus grippal B (1%).

REFERENCES

1. MILLER, I. *American journal of hygiene*, **79**: 207-217 (1964).
2. MEDICAL RESEARCH COUNCIL WORKING PARTY ON ACUTE RESPIRATORY VIRUS INFECTIONS, *British medical journal*, **2**: 319-326 (1956).
3. MUFSON, M. A. ET AL. *American journal of epidemiology*, **86**: 526-544 (1967).
4. MONTO, A. S. ET AL. *American journal of epidemiology*, **94**: 269-279 (1971).

5. MONTO, A. S. & JOHNSON, K. M. *American journal of epidemiology*, **86**: 78-92 (1967).
6. SOBĚSLAVSKÝ, O. ET AL. *Bulletin of World Health Organization*, **55**: 625-631 (1977).
7. WHO Technical Report Series, No. 642, 1980 (*Viral respiratory diseases:* report of a WHO Scientific Group).
8. VASCONCELLOS, J. V. ET AL. *Anais de microbiologia (Rio de Janeiro)*, **5**: 61-68 (1957).
9. MARQUES, A. N. ET AL. *Jornal de pediátria (Rio de Janeiro)*, **41**(11-12): 24-28 (1976).
10. MARQUES, A. N. ET AL. *Jornal de pediátria (Rio de Janeiro)*, **42**(5): 30-39 (1977).
11. MARQUES, A. N. ET AL. *Jornal de pediátria (Rio de Janeiro)*, **44**(6): 345-348 (1978).
12. THE ROYAL AUSTRALIAN COLLEGE OF GENERAL PRACTITIONERS. The Australian general practice mobidity and prescribing survey, 1969-1974. *Medical journal of Australia*, **2** (Special supplement No. 1): 18-20 (1976).
13. *Fit for the future.* Report of the Committee on Child Health Services. London H.M. Stationery Office, 1976.
14. OTA, W. K. & BANG, F. B. *American journal of epidemiology*, **95**(4): 371-383 (1972).
15. KLOENE, W. ET AL. *American journal of epidemiology*, **92**(5): 307-320 (1970).
16. FOY, H. M. ET AL. *American journal of epidemiology*, **97**(2): 80-92 (1972).
17. ONG, S. B. ET AL. *Bulletin of the World Health Organization*, **60**: 137-140 (1982).
18. *Weekly epidemiological record*, **56**(47): 374 (1981).
19. *Weekly epidemiological record*, **57**(6): 41-45 (1982).
20. CHAVES, J. R. S. ET AL. *Revista de microbiologia (São Paulo)*, **13**(2): 161-165 (1982).
21. NASCIMENTO, J. P. ET AL. *Revista de microbiologia (São Paulo)*, **13**(1): 65-69 (1982).
22. CHAKRAVERTY, P. *Archives of virology*, **63**: 285-289 (1980).
23. TOBITA, K. *Medical microbiology and immunology*, **162**: 23-27 (1975).
24. DAVIES, H. W. ET AL. *Bulletin of World Health Organization*, **56**: 991-993 (1978).
25. CHAULET, P. & KHALED, N. A. *Revue frânçaise des maladies respiratoires*, **10**: 45-52 (1982).